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(54) Title: SOLID FERMENTATION METHOD

(57) Abstract

The present invention relates to a method comprising production of a highly vital spore culture of the yeast species *Endomyces fibuliger*, also called *Saccharomyces fibuliger*. Furthermore, the invention relates to a product produced with this method. The method gives highly active material in a short time. A preferred use of the material is as a probiotic composition for animals and humans.

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# SOLID FERMENTATION METHOD

## Technical field

The present invention relates to a method comprising production of a highly vital spore culture of the yeast species *Endomyces fibuliger* also called *Saccharomyces fibuliger*. Furthermore, the invention relates to a product produced with this method.

## Background of the invention

*Endomyces fibuliger* grows like a bush of separate hyphae which under specific conditions produce so called ascospores. When growing the yeast using an ordinary fermentor with nutrients dissolved in water hyphea-like cell groupings are obtained which in dry form rapidly die when contacted with the air oxygen.

When using *Endomyces fibuliger* as a probiotic composition according to the applicant's copending SE-patent application no 9800505-1, which hereby is incorporated by reference, it is of great importance to be able to distribute the yeast by admixing it in dry feed for industrial feed production. This distribution form requires that the culture is stable during the production and storage until the consumption, with regard to temperature during the heating in the feed production and exposure to the air oxygen during storage of the premixed feed or premix. These demands are fulfilled by the spores of the yeast, but not by the hyphae-like yeast cells.

## Summary of the invention

Therefore, it was the object of the present invention to provide a method for production of highly vital spore cultures of *Endomyces fibuliger*.

The present inventor has discovered that if *Endomyces fibuliger* is allowed to grow out in solid particles of nutrient substrate with oxygen supply under suitable conditions regarding the water content in the solid material and a suitable temperature, the yeast cells will produce a layer of chalk white spores at the particle surface.

In a first aspect, the invention relates to a method for producing a highly vital spore culture by solid fermentation.

The ability of *Endomyces fibuliger* to produce spores has shown to be optimal in an environment comprised by the transition phase between the growth environment of the organism in the nutrient substrate (surface layer of the substrate particles) and the surrounding air with free access to oxygen.

Furthermore, it is required that the water content in the nutrient substrate can be kept at a certain and constant level. During the process about 20% of the dry weight of the chosen nutrient substrate is lost and transformed to heat. The heat must be diverted for the temperature in the fermentation chamber to be kept at a constant level.

Because of the dry weight loss it is required that water is drained off during the fermentation to maintain a constant water level in the nutrient substrate.

The constant water level in the nutrient substrate is achieved by allowing a continuous evaporation from the nutrient substrate in proportion to the dry weight decrease. The balancing of the water content is, for example, regulated by adjustment of the relative moisture in the air in the fermentation chamber in relation to the temperature. Alternatively, the water content may be controlled by internal heat and moisture control in the cultivation

chamber. According to the invention, a continuous production of spores is obtained until a limiting factor in the nutrient substrate has been consumed.

From an economical as well as a process technical point of view, it is of great importance to find a commercially available and cheap nutrient substrate for production of yeast spores of *Endomyces fibuliger*.

Several different marketed products (bran and groats of different grains) have been investigated. According to the invention, starch-rich substrates are used, preferably starch-rich fine fibres having an adequate amount of essential fatty acids in accordance with oatmeal. Preferably meal of peeled or unpeeled starch-rich seeds with or without addition of fine fibres and other substrate components. Unpeeled seeds have to be crushed or ground to allow access to the starch. For example, different kind of cereals such as oatmeal, rice, barley, wheat, rye, corn, durra and buckwheat, and seeds from different leguminous plants with or without additions and mixtures of suitable substrate components to the same functionality to grow out the yeast spores as for example oatmeal. Also different products from root-crops, such as potato fibres, as well as fruit products can be used.

Preferably, the following conditions are fulfilled by the nutrient substrate:
a) The raw material for the nutrient substrate shall not require any addition of other nutrient components, preferably only water.
b) Satisfying particle structure shall be obtained with respect to oxygen provision and evaporation from the material during the process.
c) Conventional methods shall be used to prepare the substrate and for admixing of the inoculum.
According to these criteria, commercially sold heat-treated oatmeal was shown to be most suitable.

The method according to the invention is not dependent on the design of the process plant.
A simple "pilot plant" comprising a well isolated cabinet with adjustable ventilation has been used for experimental production of yeast spores. A thermometer measuring the temperature in the cabinet, is via signals to a microcircuit, allowed to control an air vent the position of which is regulating the amount of air that is ventilated out from the cabinet. When the temperature rises the ventilation is increased and vice versa, so that a predetermined constant temperature can be maintained during the fermentation. Alternatively, the temperature is controlled by internal heating and cooling radiators in the cultivation chamber.

The fermentation generates a large excess of heat. For accurate control of the process, it is required that the heat can be diverted with the vaporisation from the nutrient substrate and that the relative moisture and the temperature can be controlled in relation to the incoming ventilation air. Increased moisture content developing in the material when the ventilation is too slow (amount of air/sec.) leads to saccharification of the substrate particles whereby the spore production ceases within a narrow interval. When ventilating too fast the material dries too fast and the process is discontinued. A suitable temperature and air moisture of the incoming air results in a constant moisture level in the substrate (the dry weight of which continuously decreases during the process) at that through flow of air which gives constant temperature in the fermentor cabinet.

To obtain enough air around the particles in the nutrient substrate for the purpose of providing oxygen, but also to be able to optimise the evaporation, it is required that the particle size is suitable and that the substrate is given a suitable horizontal spreading and vertical thickness. For this purpose, the nutrient substrate is applied on a fine-meshed net. Tests have shown that particle sizes with a diameter of 1-5 mm spread on a net bottom in such a

way that the height of the particles is 5-25 mm gives optimum conditions for an effective spore production when using oatmeal as nutrient substrate.

During production of spore cultures of Endomyces fibuliger, the water content in the nutrient substrate is controlled to 30-60%, preferably 40-50%. These limits are valid for, for example, oatmeal substrate. However, if material is added to the substrate which is not water- soluble then these limits are changed accordingly. The production temperature is in the range 15°C-40°C, preferably 25°C-30°C.

**Detailed description of the invention**

Below are some non-limiting examples further describing the invention.

Example 1: Preparation of nutrient substrate and admixing of inoculum

As nutrient substrate oatmeal was used, which was sterilised by a suitable method. Thereafter, sterile water was measured up so that the admixture of water into the oatmeal gave a water content in the substrate of 40-50%, preferably 45%. A starter culture of not less than 2 x $10^8$ CFU *Endomyces fibuliger*/kg of the completed substrate was mixed into the water. The oatmeal was poured in a mixer of suitable construction (of bakery machine type for the preparation of a dough). All of the water was added directly under intense mixing for a few minutes. The mixture was left and allowed to swell for about 30-60 min. Thereafter, the mixture was ground in a mill of suitable construction to produce pellets of 2-5 mm in diameter, alternatively chopped to particle sizes of 0-5 mm in diameter.

The completed inoculated solid substrate is poured in baskets with a net bottom which are put into the fermentation cabinet, wherein the baskets or cabinet is designed so that each substrate in each basket is cultivated and ventilated separated from the other baskets. The cultivation chamber can be

designed in different ways but the temperature ventilation has to be uniform over the whole horizontal surface.

Example 2: Production of spores

Preferably, cultivation is not to be performed in temperatures over 30°C but temperatures up to 40°C can be used although, at this temperature the degradation of the starch is very rapid. In the interval 25-30°C an optimum result is achieved. Also lower temperatures give satisfactory results, but not below 15°C. Because of the heat loss out through the walls of the fermentation chamber, the temperature and the air moisture of incoming air is adjusted, so that the outgoing water amount with the outgoing air corresponds to the dry weight loss in the cultivation substrate during the course of the process, totally about 20%.

In its simplest design, the method gives good results when using a) 5 cm cell plastic as an isolation in a cultivating chamber of about 1 $m^3$ to 24 kg of substrate, b) 20°C temperature of the incoming air, c) 50% relative moisture of the incoming air. When the cultivation is started the temperature can be increased up to 30°C by additional heat within the cultivation chamber. No ventilation is required before the substrate releases so much heat that cooling by ventilation is required to keep the temperature constant at 30°C. Post-treatment of the cultivation is started after about 30 hours by rising the temperature of the incoming air to 45°C at the same time as the ventilation is dramatically increased through the material in such a way that, in a first step, the water content is lowered from 45% to about 18-20%. At about 18-20% water content the material is ground in a suitable mill, and then the material is finally dried with 55°C air to 6% water content. The product is ready and contains spores of at least about 1-1.5x$10^9$CFU/g of material. To be economically interesting the product should contain at least $10^8$CFU/g.

# CLAIMS

1. A method of producing spore cultures of *Endomyces fibuliger (Saccharomyces fibuliger)* by fermentation of pre-inoculated nutrient substrate in a cultivation chamber, **characterized by** solid fermentation of the yeast in solid particles of nutrient substrate under oxygen supply and controlled temperature to 15-40°C in the cultivation chamber and controlled water content to 30-60% in the nutrient substrate, whereby the dry weight of the nutrient substrate decreases continuously without increasing its wet weight.

2. A method according claim 1, **characterized by** a nutrient substrate comprising starch.

3. A method according to claims 1 or 2, **characterized by** controlling the temperature and the water content by ventilation of air with adjustable temperature and moisture into and out of the cultivation chamber.

4. A method according to claims 1, 2 or 3, **characterized by** maintaining a constant water content in the nutrient substrate.

5. A method according to claim 4, **characterized by** providing a constant water content in the nutrient substrate by allowing continuous evaporation from the nutrient substrate by outwards ventilation in proportion to the dry weight decrease and by inwards ventilation of air having suitable temperature and air moisture.

6. A method according to one or more of the above claims, **characterized by** a temperature of 25-30°C in the cultivation chamber.

7. A method according to one or more of the above claims, **characterized by** a water content in the nutrient substrate of 40-50%.

8. A method according to one or more of the above claims, **characterized by** a substrate comprising oatmeal.

9. A method according to one or more of the above claims, **characterized by** providing the nutrient substrate with a particle size of 0-5 mm in diameter.

10. A method according to one or more of the above claims, **characterized by** spreading the nutrient substrate in a height of 5-25 mm.

11. A method according to one or more of the above claims, **characterized by** post-treatment of the cultivation material by increasing the temperature of the incoming air at the same time as the ventilation is dramatically increased through the material, so that the water content is lowered, whereafter the material is ground and then finally dried to further lower the water content.

12. A product produced by the method according to claims 1-11.

13. A product according to claim 12 comprising spores of at least $10^8$CFU/g material.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/00232

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 1/16 // A23K 1/16
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CA, BIOSIS, MEDLINE, FOODSCIENCE, BIOTECHNOLOGY ABSTRACTS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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| A | US 3294647 A (SURENDRA NATH SEHGAL), 27 December 1966 (27.12.66) -- | 1-13 |

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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Information on patent family members

07/04/99

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| US 3294647 A | 27/12/66 | NONE | |